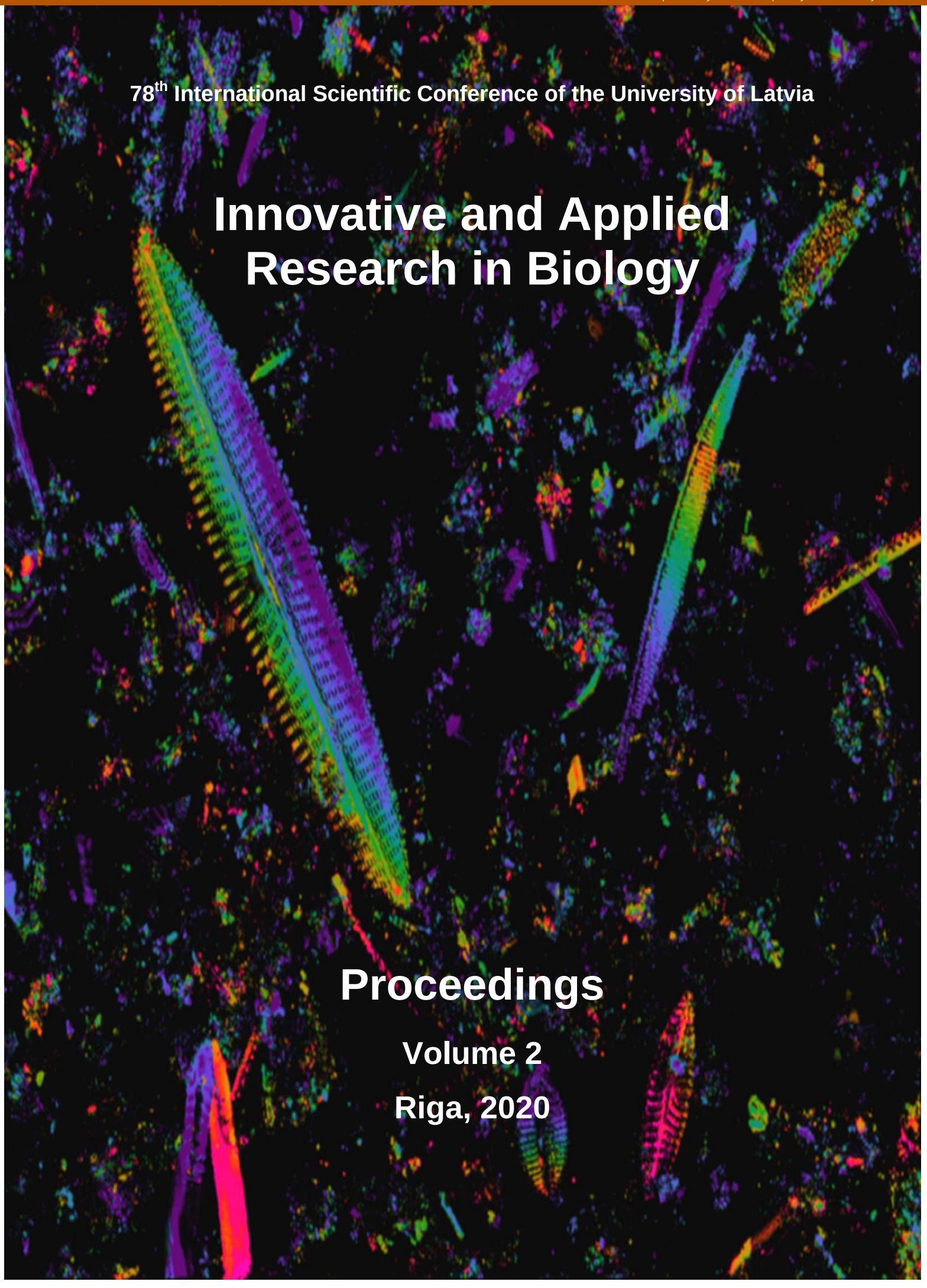

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Innovative and Applied Research in Biology

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Volume 2

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Development of nature protection plan for protected landscape area "Augšdaugava"

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***Keywords**:* protected species and habitats, cultural environment, landscape diversity

Recently were are nine protected landscape area in Latvia, and "Augšdaugava" (Upper Daugava) with 52098 ha, established in 1990 is the largest one. This area covers 10 parishes in Daugavpils and Krāslava countes inclusive the river Daugava and its valley from Piedruja to Daugavpils.

According to Latvian legislation, protected landscape areas are territories that are characterized by unique or diverse landscapes. Their aim is to protect and preserve the characteristic landscape and those landscape elements that are essential for the ecological functions of protected species and habitats, the cultural environment and landscape diversity typical of Latvia, as well as to preserve the environment suitable for public recreation and environmentally friendly management. On February 25, 2011, the protected landscape area "Augšdaugava" was included in the UNESCO World Heritage Site of Latvia. The territory includes the Nature Park "Daugavas Loki" (Meanders of Daugava), with an area of 12372 ha (Bāra, 2010), Natura 2000 sites and a significant number of nature monuments as river meanders with ravines, springs, valuable dendrological parks, alleys, lakes.

In 2019 the expert group, covering all aspects of flora and fauna species and habitats according to EU legislation performed inventory to assess the status quo of the quality and biodiversity of landscape area. The main aim is to integrate all protected units in one complex and to elaborate "a ţoad map" of management plan for the next 12 years This goal includes to optimize the living/well being, working, recreation conditions for all stakeholders in the Augšdaugava region.

More than 900 species of vascular plants have been found in the territory, of which 71 specially protected plant species, for 33 species micro-reserves wil bet o established. In "Augšdaugava", nests 38 bird species included in Annex I of the Birds Directive (2009/147/EC), as well as 16 bird species that are specially protected only in Latvia. 22 specially protected insect species have been identified at the national level and 10 insect species at the European level, micro-reserves will be established for 4 species. There are 20 species of molluscs of nature protection significance in the area. Four of them are EU protected species. DP "Daugavas loki" is the only place in Latvia where the snail Isognomostoma isognomostomus is found. There are four protected species of fish and one species of lampreys. The area is important for the conservation of bat species, especially the pond bat Myotis dasycneme.

AAA "Augšdaugava" DA plan is developed within the framework of the EU Cohesion Fund project "Creation of Preconditions for Better Conservation of Biodiversity and Protection of Ecosystems in Latvia" and is financed by Nature Conservation Agency.

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Identification and application of informative genetic markers in studies of genetic diversity of experimental and wild populations of *Lemna minor*

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Abstract: Investigation of intraspecific genetic diversity of experimental and wild populations of duckweed (*Lemna minor*) using different genetic markers revealed the most informative and sensitive markers that could be further applied to detect nucleotide substitutions in nuclear or chloroplast DNA. Comparison of the naturally developed genetic variability detected at three microsatellite loci L4, L14, and L16 of chloroplast DNA and some selected fragments of nuclear genes revealed that the percentage of genetic variability attributed to the inter-population diversity identified using microsatellite markers of chloroplast DNA was higher (30% (L4), 29% (L14), 33% (L16)) than the same genetic parameter calculated for fragments of antioxidant genes (5% (GPx6), 10% (GPx7), 15 % (Cat4-Cat4b), 10% (Cat7), 19% (APx1-APx2)) of nuclear DNA. It was also found that the growth of experimental clone of *L. minor* was suppressed after 14th week from the beginning of the exposure to low-frequency (50 Hz) electromagnetic radiation (LFER) and new nucleotide variations in some fragments of antioxidant genes GPx, Cat, and APx were detected after 14th and 18th weeks exposure to LFER.

Keywords: *Lemna minor*, genetic diversity, chloroplast microsatellite markers, GPx, Cat, APx gene sequencing, electromagnetic field.

Introduction

Duckweed (*Lemna minor*) is a free-floating freshwater macrophyte and one of the most suitable plants to test the environmental toxicity of chemical contaminants in higher aquatic plants (Park et al., 2013). Such biological effects, like growth inhibition due to radiotoxicity or induction of oxidative stress after chronic gamma radiation of *L. minor* clones that induced altered polyploidy level, were studied intensively (Van Hoeck et al., 2015). Therefore, such issues like induction of DNA polyploidy of *L. minor* cells could affect the results of genetic analysis after investigation of the impact of radiation on duckweed as genotoxic stressors usually induce endoreduplication events in plants (De Veylder et al., 2011).

Although the duckweed has become a popular model plant species in the studies of genotoxic effects the impact of low-frequency electromagnetic radiation (LFER) on the growth parameters and genetic diversity in experimental clones or wild populations of *Lemna minor* remains abundant. Therefore, the aim of the current study was to reveal the genetic diversity of wild populations of *L. minor* using different genetic markers and to select the most informative and sensitive markers that could be further applied to detect nucleotide substitutions in nuclear or chloroplast DNA comparing fragments representing naturally developed or experimentally induced genetic

variability by sequencing some parts of uncoding chloroplast DNA and fragments of GPx, Cat and APx genes before exposure and after prolonged exposure to LFER plants grown in specific conditions.

Materials and methods

Representatives of the wild population consisted of wild-type *L. minor plants* collected from Nemunas River near Kaunas (4 specimens) and Neris River near Vilnius (8 specimens).

All collected plants were screened and the most suitable colony was selected to be kept as a laboratory clone based on the results of DNA profile purity after sequencing of different fragments of antioxidant genes GPx, Cat, and APx. The laboratory clone for growing in sterilized modified Steinberg medium for further experimentations was entitled Sta2. Experiments were carried out in the same chamber at 25°C under a 16/8-h light/dark cycle. OSRAM L 36/77 Fluora, 100-120 $\mu E \cdot m^{-2} \cdot s^{-1}$ light intensity that was held during the lighted part of the cycle. In the current experiment, the environment with low-frequency electro*magnetic radiation (LFER)* was generated using Helmholtz Coil, the diameter of coils was about 50 cm, the distance between coils - 27 cm, electromagnetic field (EMF) frequency was ~ 50Hz, magnetic field inside the coils was nearly uniform and reached 650 μT.

The genetic diversity of the wild type *L. minor* was studied using the genetic markers of the chloroplast genome including microsatellite sequences surrounded by non-coding regions (microsatellite loci L4, L14, and L16) and selected fragments of nuclear antioxidant genes (GPx, Cat, APx).

The growth parameters like intensity and frond area were compared and DNA sequences of genetic markers were obtained after growing *L. minor* clones in Petri dishes placed at different distances from the source of electromagnetic radiation.

Results and discussion

Overall, seven genetic markers (GPx6, GPx7, Cat4b, Cat4b, Cat7, APx1, APx2) were identified as suitable for wild type and experimental *L. minor* clones genetic diversity studies. Comparison of the naturally developed genetic variability detected at three microsatellite loci L4, L14, and L16 of chloroplast DNA and selected fragments of nuclear genes revealed that the percentage of genetic variability attributed to the inter-population diversity identified using microsatellite markers of chloroplast DNA was higher (30% (L4), 29% (L14), 33% (L16)) than the same genetic parameter calculated for fragments of antioxidant genes (5% (GPx6), 10% (GPx7), 15 % (Cat4-Cat4b), 10% (Cat7), 19% (APx1-APx2)) of nuclear DNA. It was also found that the growth of experimental clone of *L. minor* was suppressed after 14th week from the beginning of the exposure to low-frequency (50 Hz) electromagnetic radiation (LFER) and new nucleotide variations in some fragments of antioxidant genes GPx, Cat, and APx were detected after 14th and 18th weeks exposure to LFER.

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De-icing salt contamination in street greeneries: responses of lime trees (*Tilia* sp.) and impact on the arthropods' communities

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Keywords: street trees, NaCl, ecophysiology, foliage injury, *Eucallipterus tiliae*

Urban ecosystems, including street greeneries, provide various ecosystem services, including decreased air and soil pollution, improved microclimate, reduced runoff, or improved biodiversity. However, these services can be adversely influenced by various anthropogenic factors. In cities located within the boreo-nemoral and other high-latitudes climatic zones, having air temperature below zero Celsium during the winter season, large quantities of de-icing salts are applied on roads and sidewalks to prevent ice formation (Marosz, 2011, Equiza et al., 2018). The most common de-icing material used in many countries is sodium chloride – NaCl (Dobson, 1991, Ordóñez-Barona et al., 2018). The use of a high amount of de-icing salts on pavement leads to its deposition in the surrounding environment during the following vegetation seasons (Bryson, Barker, 2002, Cekstere et al., 2008, Ordóñez-Barona et al., 2018). Salt contaminants can affect not only the soil and groundwater but also the vitality of street trees and greenery (Czerniawska-Kusza et al., 2004; Dmuchowski et al., 2019). Lime trees (*Tilia* sp.) belong to the most often planted and widespread tree ornamentals in city street greeneries such as that of Riga, Latvia.

The overarching aim of our research has been to assess the impact of de-icing salt (NaCl) on lime trees and arthropod communities in foliage, either in the field, along a gradient of salt contamination in the street greenery of Riga (Latvia), or in controlled conditions, in the framework of a common garden experiment with potted young trees exposed to deicing salt in partly controlled conditions.

The exposure to elevated levels of NaCl in the soil substrate triggered the formation of functional and structural injuries within the foliage of *T. cordata, T. platyphyllos,* and *T.* x *vulgaris* and reduced the development of lime aphid (*Eucallipterus tiliae*) colonies (Bouraoui et al., 2019). Generally, the three treated lime tree species showed contrasted tolerance to salt exposure during 2015-2019. Significant alterations in the soil physico-chemical parameters as well as changes in the composition/abundance and diversity of arthropod communities, e.g., Homoptera, Diptera, Hymenoptera, etc. were observed at the salt polluted *versus* salt unpolluted research sites of Riga's street greenery. Typical lime aphid injury in foliage – *i.e.* the production of pectin-like mucilage substances in leaf epidermis and intercellular spilling within mesophyll – showed decreased severity as a function of increasing foliage contamination with NaCl, in line with severe reduction of lime aphid colonies.

Hence, the application of deicing NaCl salts can have far-reaching as well as cascade effects within urban ecosystems, negatively affecting not only the soil and

trees but also the trophic chain and the arthropod communities, especially its aphid main herbivore component. Ongoing screening efforts, as to identifying better salt-tolerant tree cultivars and species with a view to improved street greenery sustainability, need to also consider the consequences on the provided urban ecosystem services - including the urban biodiversity - globally.

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Trends in numbers of autumn migration of birds at Baltic Sea coast at Pape, Latvia 1992–2019

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Keywords: *Heligoland trap, long-term data on bird migration*

Observations of autumn migration at the Baltic Sea coast in Pape village has been conducted first in 1958 (Mihelsons et al., 1960), the capture of birds in Pape started in 1966 (Blūms et al., 1967), but in standardized way in small Heligoland type trap – since 1992. We analyzed long-term data (1992-2019) of the annual number of birds (mostly Passerines) captured in Pape Heligoland trap for 27 species using TRends for Indices and Monitoring (TRIM) software (Pannekoek & van Strien, 2005).

A decrease of numbers in the period of 1992–2019 was observed for 12 species: Blue Tit *Parus caeruleus*, Blackcap *Sylvia atricapilla*, Willow Warbler *Phylloscopus trochilus*, Garden Warbler *Sylvia borin*, Chiffchaff *Phylloscopus collybita*, Sparrowhawk *Accipiter nisus*, Lesser Whitethroat *Sylvia curruca*, Great Tit *Parus major*, Redwing *Turdus iliacus*, Siskin *Carduelis spinus*, Goldcrest *Regulus regulus,* and Wren *Troglodytes troglodytes*.

A stable trend was observed for 9 species: Willow Tit *Parus montanus*, European Robin *Erithacus rubecula*, Coal Tit *Parus ater*, Lesser Spotted Woodpecker *Dendrocopos minor*, Pied Flycatcher *Ficedula hypoleuca*, Song Thrush *Turdus philomelos*, Spotted Flycatcher *Muscicapa striata*, Common Treecreeper *Certhia familiaris,* and Chaffinch *Fringilla coelebs*. Two other species showed an increase over the years 1992– 2019 Common Redstart *Phoenicurus phoenicurus* and Blackbird *Turdus merula*.

There is an unclear trend for 4 species which are irruptive species, who's increase is depending on mast seeding years (Hildén 1977): Greater Spotted Woodpecker *Dendrocopos major*, Long-tailed Tit *Aegithalos caudatus*, Bullfinch *Pyrrhula pyrrhula,* and Crested Tit *Parus cristatus*, these species are showing large variation between years, e.g. Long-tailed Tit from 0 (years 1994, 2011) to 22227 (the year 2000) captured individuals.

Long-term data on bird migration can provide valuable insights on phenology and impacts on climate change on bird populations over considerable time and geographical extent when collected and analyzed from many bird observatories like it is done in the analysis by A. Lehikoinen and others (2019) on spring migration. Similar analyses on autumn migration in Europe are still a task ahead of us. Paper archives of many bird observatives, including Pape (since 1966) is an obstacle to fully analyze data at the moment.

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Development of methods for biotextile testing

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Abstract: Properties of biotextile materials such as protective ability against UV-B radiation and reduction of electromagnetic field impact/effect were tested exploring two different model-species (*Lemna minor* and *Drosophila melanogaster)*. Evaluation of speed growth and induction of SNP in *Lemna minor* DNA derived from the affected plants experimentally grown *in vitro* cultures and mortality rate at the post-embryonic development indicated as survival test of the fruit flies (*D. melanogaster*) *has been demonstrated to be informative and sensitive methods for biotextile testing*.

Keywords: biotextile, *Lemna minor*, *Nicotiana tabacum*, *Drosophila melanogaster*, UV-B radiation, electromagnetic field

Introduction

It was found that *in vitro Lemna minor* cultures usually contain symbiotic forms such as bacteria and algae that coexist with *L. minor in vivo*, and the presence of the symbionts could have an influence on the results of genetic investigations (Glick 2012; Ishizawa et al. 2017; Thomson, Dennis, 2013). Therefore, obtaining an axenic *Lemna minor* L. line is essential to get pure DNA derived from a single *L. minor* species. The Survival test of *Drosophila melanogaster* L. affected by ultraviolet (UVB) irradiation experimentally induced by 365 nm intensity lamp was conducted as well to assess the capacity of textile materials with incorporated amber yarn protect tested organism from negative impact of ultraviolet (UVB) irradiation. The cells cultures of Lemna minor and *Nicotiana tabacum* for ascertaining of cell reaction changes after the exposition of cell cultures in the low frequency (50 Hz, 400 μT) electromagnetic field (LF EMF) were used for the elaboration of methods for detection of biotextile protective properties against LF EMF.

Material and methods

To obtain viable axenic lines of *Lemna minor (*Common Duckweed), several plants representing wild type colonies were subjected to a multi-step pre-treatment before further cultivation *in vitro*. Plants first treated with 10% $KMnO_4$ solution for 10 seconds and rinsed into deionized H_2O followed by 10 seconds treatment with 70% C_2H_5OH and washed with deionized H_2O repeatedly. After rinsing, the plants displayed on Petri dishes with Steinberg medium were prepared according to the ISO 20079 and supplemented with sucrose 10 mg/L. The plants cultivated in climate chambers with a photoperiod of 16h light / 8h dark. After 5 days from the collection in a wild

environment, the plants transferred from natural water to Steinberg medium and cultivated as described before. The efficiency of treatment checked by microscopy and flow cytometry.

The confocal laser scanning microscopy was applied to evaluate the anatomic structure of *Lemna minor* L. and *Nicotiana tabacum* L. cells. Morphologic variables were evaluated to characterize the possible impact of EMF on tested cells after it's shielding by covering of Petri dished with tested cells grown in a specific medium with different specimens of biotextiles. More than 20 knitted samples with and without amber particles tested regarding properties modifying impact of EMF on living cells.

To obtain eggs for "Survival test" of *Drosophila melanogaster* (Weisman et al., 2014) line, Canton-S Jazz-Mix food with concentration 18,9g+100mL H_2O was used. The medium placed into Petri dishes (Ø 6mm) compatible with embryo obtaining cage lids. After copulation, 3-7 days old *D. melanogaster* males and females were placed in the egg-laying cages. *Drosophila* eggs (each size around 0.5 x 0.2 mm) were collected and placed in tubes (50 eggs in each tube) on the medium and cultivated at 25 °C. After 4 days the larvae are rinsed out of the medium and transferred onto Petri dishes with 5% sucrose and 0.9% NaCl solution 30 larvae are placed in one Petri dish (6 mm) with 3 ml of solution.

In order to determine the protective performance of different biotextile fabrics UVB lamp was placed 30 cm above the table's surface (at 365 nm intensity). The larvae of *D. melanogaster* were irradiated with UV keeping them in closed Petri dishes covered with different textile specimens made with incorporated and without amber yarn. Besides tested fabrics, a positive control (uncovered Petri dish) was used. The exposure to UBV irradiation was continued 60 and 75 minutes, for two groups of tested larvae, respectively.

After being worked on, from the Petri dish's the 5% and 0.9% solutions are removed and the larvae are transferred to tubes with a medium. After 6-8 days, the pupa and imago of *D. melanogaster* counted.

The flow cytometry-based methods (Grauda et al, 2015) for ascertaining cell reaction changes after growing of test cultures wrapped into biotextile specimens and the comet assay based on examination of tobacco *Nicotiana tabacum* cell nucleus could be sensitive enough to explore properties of biotextile to protect cells from DNA damages that could be caused after the exposition of cell cultures in the low frequency (50 Hz) electromagnetic field (EMF).

Results

It was found that after incubation of *L. minor* colonies in 50 Hz, 400 μT Electromagnetic Field (EMF) possible changes of the parameters reflecting the impact on life processes is possible to determine using flow cytometry-based cell sorting method (Grauda et al., 2015).

A scorable changes of cell fluorescence were found after 2h incubation in EMF. One type of knitted textile containing amber micro particles was identified as material effectively protecting cells from the impact of 50 Hz, 130 μT EMF as the structure of cells didn't changed in comparison to the absence of protective properties of all the rest biotextile specimens.

The highest survival percentage found in the group of larvae covered with textile that encompassed amber yarn. The survival of larvae that developed to imago in the group of *D. melanogaster* covered with textile without incorporated amber was lower. No one larvae developed to imago in both control (uncovered) groups treated with UVB.

The survival test of *D. melanogaster* demonstrates that the amber yarn increases the protective capacity of biotextile against Ultraviolet (UVB) irradiation enhancing the percentage of surviving larvae up to 20% (Figure1).

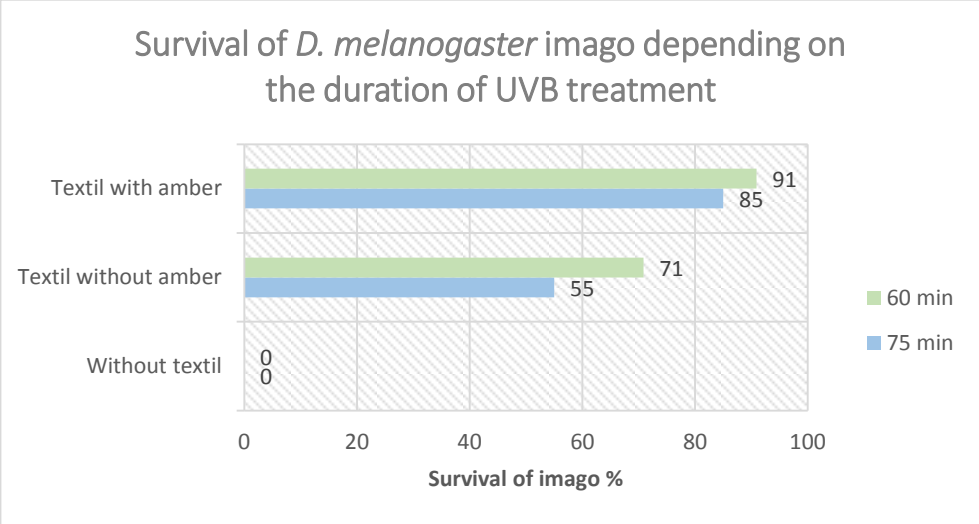

Figure 1. Survival of *D. melanogaster* imago depending on the duration (60 or 75 min.) of UVB treatment.

The flow cytometry-based methods (Grauda et al, 2015) and comet assay based on examination of tobacco *Nicotiana tabacum* cell nucleus are found as useful for biotesting of biotextile properties to protect cells.

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Adaptation of techniques for testing antimicrobial properties of amber nano and micro size particles

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Abstract: The amber nano and micro size particles are being studied as potential raw material for integration in synthetic and natural fibres used to produce new biotextiles materials. The aim of the study was to adapt methods and techniques for determination of impact of amber (succinate) nano and micro size particles on bacteria and microscopic fungi *in vitro*. We used reference strains of fungi *Aspergillus niger, Chaetomium cochliodes,* and bacterias *Escherichia coli, Staphylococus aureus,* and *Enterococcus faecalis.*

Keywords: succinate, bacteriostatic effect, fungistatic effect, *Aspergillus niger, Chaetomium cochliodes, Escherichia coli, Enterococcus faecalis*

Introduction

Amber or succinate ($C_{40}H_{64}O_4$) is a fossilized wood resin formed from coniferous or succulent exudates (mainly pine *Pinus succinifera* Göppert) and matured during the Palaeogene period by polymerization, disposal of volatile components in the evaporation process - polycondensation of terpenes and resin acids, as well as isomerization (Gaidukovs et al., 2016). Amber has been appreciated for its colour and natural beauty since Neolithic times (Grimaldi, 2009). Amber has been used in folk medicine for many centuries, using nuggets in jewellery or using various tinctures orally and externally, believed to help diseases such as rheumatism, epilepsy, and others, as well as amber-based various cosmetics and pharmaceuticals (Matuszewska, 2010). However, the beneficial effects of such products are questionable, as there are no validated scientific studies indicating a positive effect of amber on the human body (Tumiłowicz et al., 2016).

The growth of microorganisms in the textile materials causes innumerable problems such as unacceptable odour, loss of strength in fabric, stains and, moreover, affect the health of the wearer. It is therefore important that the antimicrobial effect on textiles is being studied to protect the user's health (Gokarneshan et al., 2012). The antimicrobial effects of different types of nano-materials have been studied and are expected to play an important role in medicine. Their effects on human health are being thoroughly tested for commercial approval (Gokarneshan et al., 2012). Amber is considered a potential protective agent. The amber nano and micro size particles are being studied as potential raw materials for integration in synthetic and natural fibres used to produce novel bio-textiles materials.

The general aim of the project was to determine the impact of newly developed innovative biotextile and its compounds (amber particles) on procariots. The aim of this study was to adapt methods and techniques to determine the antimicrobial properties of amber.

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Material and methods

Amber particles

Suspensions of amber particles of two fractions (fine <1 μm and coarse <3 μm) were used in the experiments. Water suspension of amber particles (5%) was prepared. To prevent particles from sticking, Twin 20 was added to the suspension (0.01 %). Our previous studies showed that the amber particle powder contains microorganisms (Jankevica unpublished), therefore sterilization (15 min 121 °C) of the amber suspension was done. There is no information in the literature on the negative effect of temperature on the bioactivity of amber (Halbwachs, 2019).

Reference cultures

Fungal strains *Aspergillus niger* van Tieghem, LMCC #324 and *Chaetomium cochliodes* Palliser, LMCC #1536 obtained from the Latvian Microorganism Culture Collection (LMCC), stored in liquid nitrogen at -196 °C were used in experiments. In the laboratory fungal strains were maintained on potato dextrose agar (PDA) medium (SIFIN, Berlin). *A. niger* and *C. cochliodes* were grown on PDA for ten days before being used in the study.

Bacterial strains *Escherichia coli* (Migula 1895) Castellani and Chalmers 1919, LMCC #332*, Enterococcus faecalis* (Andrewes and Horder 1906) Schleifer and Kilpper-Balz 1984, LMCC #302, and *Staphylococcus aureus* subsp. *aureus* Rosenbach 1884, LMCC #334, obtained from the Latvian Microorganism Culture Collection, stored in liquid nitrogen at -196 °C were used in experiments. In the laboratory, bacteria were maintained on TSA.

In vitro mycelial growth inhibition assay

The growth of fungal mycelium due to the additional suspension of amber particles at various concentrations was determined using the radial growth method. Amber particle suspension were added to sterilized PDA to obtain final dose 0.001; 0.005; 0.01; 0.05; 0.1 mg/ml or spread on PDA agar surface in concentrations of 0.0001; 0.001; 0.01; 0.1; 1 mg/cm^2. Agar plugs (5 × 5 mm) from pure cultures of fungi were placed in the centre of 90 mm Petri dish with PDA and amber particles in various doses. As a negative control PDA with 1 mg/ml Bordeaux mix ($[Ca(OH)_2]*CuSO_4$, produced by Karaleks Ltd., Latvia) was used and as the positive control - PDA without any additions. Each treatment was replicated five times. Inoculated Petri dishes were incubated at 25 ± 2 °C in the dark. Radial growth of the mycelium in two directions was measured daily for ten days or till the fungus had reached the edge of the dish. Antifungal activity was expressed in terms of percentage of mycelial growth inhibition at each dose and calculated according to the following formula: P (%) = [DC- DT] / DC] × 100, where P = mycelial growth inhibition (%); DC and DT are the average diameters of fungal colony of control and treatment, respectively (Pandey *et al*., 1982; Zambonelli *et al*., 1996).

Determination of antibacterial properties

We tested the effect of amber particles on the growth of bacteria using Ecometric method (absolute growth index (AGI) and relative growth index (RGI) were calculated) and surface inoculation technique (productivity ratio (P.R.) were calculated) (LAB M, 2002). A controlled inoculum of approximately 100 colony forming units (CFU) was spread on control media Tryptic Soy Agar (TSA) and TSA with addition amber particles

<1 μm and <3 μm in concentrations (0.005; 0.01; 0.025; 0.05; 0.075; 0.1; 0.2 mg/ml). The P.R. is calculated by counting the CFU on the test and control media.

Statistical analysis

The means of colony diameters were separated using Least Significance Different Test, at *P*< 0.05.

Results and discussion

In vitro mycelial growth inhibition assay

Pouring or spray of succinate particles on PDA media affected the growth of *A. niger* and *C. cochliodes.* Using pouring amber particles (fine fraction (<1 μm) in concentrations 0.01 – 0.1 mg/ml after two days cause significant (*P<0.05*) mycelia growth inhibition of *A. niger* (21.3 - 25.0%) and *C. cochliodes* (16.7 - 18.8%) (Table 1).

Table 1. Inhibition of mycelial growth of *Aspergillus niger* and *Chaetomium cochliodes* isolates recorded two and three days after inoculation on PDA without and with succinate particles in different doses (mean ± standard deviation).

Method	Dose	Inhibition of mycelial growth (P) (%)							
		Aspergillus niger				*Chaetomium cochliodes*			
		2 days		3 days		2 days		3 days	
	mg/ml								
Addition of particles (<1 μm) to nutrient medium	0.001	4.0 ± 2.3	a	2.9 ± 2.0	a	4.5 ± 1.4	a	5.1 ± 2.4	a
	0.005	5.3 ± 2.6	a	7.6 ± 1.6	ab	11.0 ± 3.6	b	8.5 ± 2.5	ab
	0.01	**21.3 ± 6.1**	cd	17.1 ± 1.9	d	11.9 ± 2.4	b	**20.0 ± 4.1**	c
	0.05	**24.0 ± 4.0**	d	**23.9 ± 2.9**	e	**18.8 ± 2.7**	c	**23.8 ± 2.9**	c
	0.1	**25.0 ± 2.8**	d	11.4 ± 2.8	c	**16.7 ± 2.4**	c	NI	
Addition of particles (< 3 μm) to nutrient medium	0.001	NI		NI		NI		NI	
	0.005	NI		7.3 ± 2.4	ab	NI		NI	
	0.01	12.3 ± 1.8	b	2.4 ± 1.9	a	8.4 ± 1.5	b	NI	
	0.05	9.4 ± 2.4	b	5.2 ± 1.6	a	17.9 ± 2.4	c	NI	
	0.1	5.3 ± 2.4	a	NI		11.6 ± 2.4	b	NI	
	mg/cm^2								
Surface inoculation (<1 μm)	0.0001	NI		11.5 ± 4.3	c	NI		NI	
	0.001	8.1 ± 2.4	ab	18.0 ± 1.6	d	8.7± 1.0	b	10.4 ± 2.5	b
	0.01	9.7 ± 2.8	ab	17.5 ± 2.5	d	17.9 ± 4.4	c	18.5 ± 2.5	c
	0.1	17.7 ± 4.2	c	20.2 ± 0.9	d	37.5 ± 2.1	e	31.9 ± 4.4	d
	1	**39.5 ± 2.4**	**e**	**38.3 ± 0.9**	**f**	**44.9 ± 2.4**	**f**	**46.7 ± 2.2**	**e**
Surface inoculation (< 3 μm)	0.001	NI		NI		NI		NI	
	0.01	2.5 ± 1.2	a	NI		12.4 ± 1.4	b	NI	
	0.1	12.2 ± 2.4	b	14.6 ± 1.8	c	11.2 ± 2.5		17.0 ± 3.2	
	1	15.7 ± 2.9	bc	16.2 ± 3.2	c	**30.7 ± 1.5**	**d**	**31.0 ± 3.2**	
Bordeaux mix	0.1 **mg/ml**	**95.0 ± 0.5**	**f**	**88.6 ±1.4**	g	**95.0 ± 0.5**	g	**86.4 ± 1.3**	

Means with the same letters within columns are not significantly different at *P*<0.05.
NI - inhibitions of fungal growth was not observed; x- not tested.

Mycelia growth inhibition of. *C. cochliodes* and *A. niger* caused by the concentration of succinate particles (<1 μm) 1 mg/cm^2 after two and three days are in the range 44.9 – 46.7% and 38.3 - 39.5 %, respectively (Table 1.). Using lowest concentrations, we did not observe significant inhibition of *A. niger* and *C. cohliodes* mycelia growth. After 5 - 6 days, the mycelia growth did not differ from control. This allows us to conclude that the addition of amber has a fungistatic impact on mycelia growth in the first three days.

The experiments with a coarser fraction showed a significantly (*P <0.05*) lower inhibition coefficient than a fine fraction, and in some cases, no differences from control were observed. This could be explained by the fact that the coarser particles settle faster in Petri dishes after pouring the medium and a small amount of particles come into contact with the fungi.

The high inhibitory effect of amber micro and nano particles (inhibition more than 50%) was not observed using pour plate and spread methods. Obtained results showed that the use of the spread method shows greater mycelial inhibition than the addition of particles to the medium.

Determination of antibacterial properties

No significant impact of the addition of amber particles on absolute growth index for bacteria. *Escherichia coli*, *Enterobacter faecalis* and *Staphylococcus aureus* were observed. Relative growth index was in the range 95.0 – 96.3% and did not show significant inhibition of bacterial activity caused by the addition of succinate particles in tested concentrations to nutrient media.

The total number of bacteria *Escherichia coli, Enterobacter faecalis,* and *Staphylococcus aureus* using the surface inoculation method did not differ significantly between the control and the variants with the addition of amber particles (Figure 1).

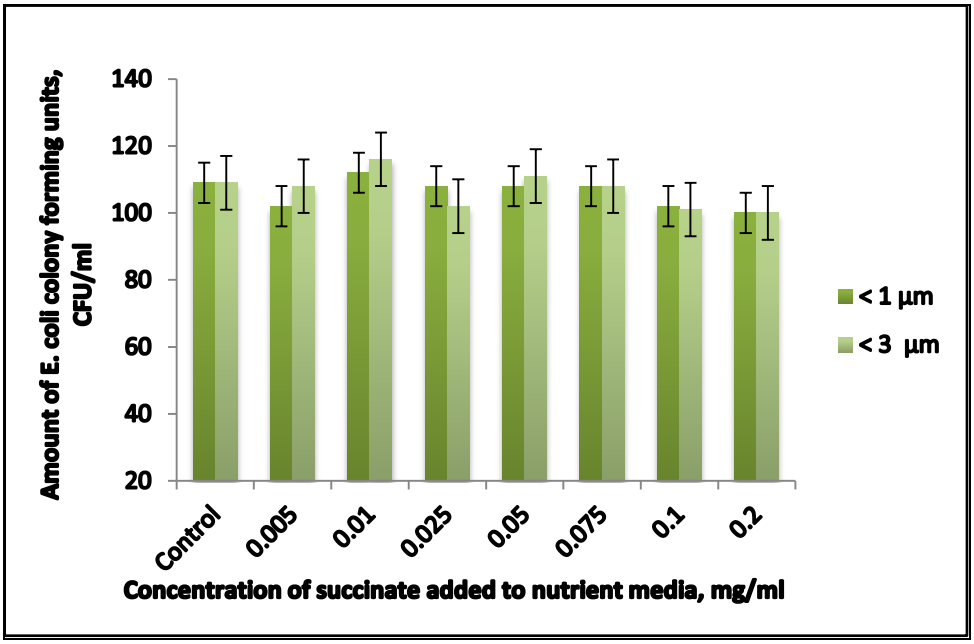

Figure 1. Total number of *Escherichia coli* colony forming units 72 h after surface inoculation on control (TSA) and TSA medium with addition of amber particles in various concentrations.

Calculated productivity was the range from 91.2 to 109.2 %. No significant differences were observed between variants where fine and coarse particles were added to nutrient media

We observed that colonies of *E. faecalis* on TSA medium with the addition of succinate particles in concentrations higher than 0.05 mg/ml are smaller and grows slowly.

The performed experiments showed that the chosen methods are not sensitive enough to evaluate the antibacterial properties of amber particles. In the future, it is planned to adapt the flow cytometry and microplate method to determine antibacterial properties.

Gaidukovs and colleagues (2016) reported that succinate have an extremely high number of organic compounds of enormous chemical diversity, therefore evaluation of the effect of different size particles on microorganisms is necessary. Although this study presents promising results on antifungal properties of succinate, further investigations on the practical applicability in biotextile producing are required.

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Edible coatings for berries: an innovative, perspective and environmentally-friendly approach to berry storage

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Keywords: extracellularly produced polysaccharides; edible coatings; biodegradable films; shelf-life; fruit quality.

Over the last decades, the world's population has grown significantly, so the issue of innovative food product development and enhancing the quality of already existing ones is becoming increasingly relevant. There is a strong correlation between life expectancy and functional food consumption. Today, a healthy lifestyle and a balanced diet are a modern trend for which consumer demand is steadily increasing. Food products must meet not only the quality standards, but they should be with high-added-value, e.g., containing no artificial supplements and shall possess positive health benefits. Strawberries and raspberries among the berries frequently used in the diet are highlighted as the primer source of various phytochemicals, including, vitamin C, anthocyanins, and phenolic acids (Mazzoni et al., 2020; Pantelidis et al., 2007). Despite the positive profile and well-documented health benefits, strawberries, and raspberries are considered highly perishable and seasonal products, so the availability of these berries on the market is limited. For postharvest shelf-life extending purposes the use of appropriate storage, and/ or pre-treatment types that would help to maintain berry quality throughout the entire storage life need to be identified. Among the storage techniques used, such technologies as ULO (Ultra-low oxygen), ULO-type bags, Janny MT storage modules, ozonation have shown to be effective. However, the above-mentioned technologies are reported to have some drawbacks which significantly affect the sensory profile of stored products and, besides, require additional energy and material input, which makes them not economically feasible. The use of such edible coatings as shellac wax (E 904), candelilla-shellac wax (E 902), or carnauba-shellac wax (E 903) formulations were found to be effective in preserving fruit and berry quality aside from microbiological safety ensuring superior sensory properties. It is worth noting, though that for the production of shellac, lac resin which is exclusively secreted by the lac insects of the family *Kerriidae* (*Metatachardia*, *Kerria*, *Laccifer*, *Tachardiella*, *Austrotachardiella*, *Afrotachardina*, *Tachardina*) so the production costs are relatively high as well as the availability of resources on the market is limited (Takumasa, et al., 2007). More recent studies have shown that such extracellularly produced microbial polysaccharides as xanthan (E 415), dextran, curdlan (E 424), and levan are promising polymers that are widely used in the food production sector as emulsifiers, stabilizers, texturizers, and sweeteners (Qureshi et al., 2020). However, the application of these polymers as edible coatings for berries, especially soft types are relatively rare documented.

The aim of the study: The limited information on the ability of extracellularly produced microbial polysaccharides (specifically, levan) to serve as edible coating promoted the design of this work, addressing the production and characterization of microbially produced exopolysaccharide levan with its further exploitation as an edible coating and film for maintaining the quality of strawberries and raspberries and extending their shelf-life.

Acknowledgements

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Influence of the long-time exposure of 50 Hz electromagnetic fields on the duckweed (*Lemna minor* L.) growth

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Abstract: There is still lack of investigations of EMFs effects on plants. In this work the effect of week power EMFs on growth and development of duckweed (*Lemna minor* L.) was analysed.

Keywords: low-frequency electromagnetic field, model organisms, lesser duckweed, relative plant growth

Introduction

The effects of low-frequency electromagnetic fields (EMF) on plants have not been studied intensively, especially, continuous effect of EMF over a long period of time. Plants are essential components of a healthy ecosystem. Many of them are easy to grow in controlled laboratory conditions. Therefore, they can be useful in experiments as model organisms (Magone, 1996; Aman & Ramneek, 2014). Lesser duckweed (*Lemna minor* L.) is a widely used model organism in studies of influence of various factors, since it is possible to assess the effect of factor on the whole organism (ISO 20079). In this study the effect of EMF on growth and development of *L. minor* is analysed. Instrumentation for controlled exposure to EMFs was designed at the Institute of Biology, the University of Latvia.

Materials and methods

L. minor line 'BOLD4' was used to detect the effects of a low-frequency EMF. At the beginning of the experiment, one plant (colony) was placed in each Petri plates (35 x 10 mm with caps, sterile) with 5 mL of Steinberg medium (ISO 20079). In total 20 Petri plates with *L. minor* plants were prepared and were exposed to continuous exposure influence of the EMF of 50 Hz with density of 1 μT. Similar 20 plates were used as a control. All samples were cultivated under controlled conditions (temperature 24 °C, 16/8 hours light/darkness photoperiods) in the climate chamber.

EMF field was created by a copper wire coil with diameter of 200 mm and height of 100 mm. To power the coils an alternating current generator was used along with a digital processor to control the current. To measure and control of the density EMF magnetic component the Three-axis Hall Magnetometers THM1176-LF ("Low Field" model, MetroLab, Switzerland) was used.

Total duration of the experiment was 42 days. Every seven days all plants were moved to a fresh Steinberg medium, and all samples were photographed to record changes of plant's growth. Area of the plant's frond was measured with the software ImageJ, and obtained data was used for calculation of relative growth of plants (Tkalec et al., 2005). The resulting values of samples subjected to EML were expressed as a percentage of the value of the control (data obtained the same day). The significance of the results was evaluated by T-test ($P < 0.05$).

Results and discussion

We observed that the influence of EML on the growth of *L. minor* depended on the duration of the exposure At the first two experiment stages (day 7th and 14th) an increased effect on plant growth was observed - the relative plants growth of exposed plants was 9.3% and 14.7% higher than control, respectively. After three weeks and later, a negative effect of EMF on growth of *L. minor* was observed - relative plant growth was 34.1% (day 21st), 21.4% (day 28th), 13.4% (day 35th) and 32.1% (day 42nd) slower than control.

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Populations of cloudberry (*Rubus chamaemorus* L.) as a potential source of antioxidants

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Abstract: The biochemical composition of cloudberries varies depending on the growth zone and climatic conditions. In biochemical tests, we used materials from the Belarussian subpopulation of *Rubus chamaemorus.* Total antioxidant activity (AA) from extracts of leaf blades is higher than that of petioles. The highest AA observed in the northernmost and shady conditions coenopopulation, which consists of old, generatively active clones. The smallest AA is characteristic of the young population growing on the site of a burnt area in an open sun exposed area.

Keywords: *Rubus chamaemorus,* coenopopulations, peroxidase activity, protein concentration

Introduction

Cloudberry is a relict perennial plant species of periglacial flora, widespread in the circumboreal zone of the northern hemisphere (Ehrich *et al*., 2008). Cloudberry's typical habitats are raised bogs and wetlands. In the Republic of Belarus cloudberry populations are located on the southern border of its range in habitats 7110*, 91D0 (Пугачевский, 2013). The taxon is Included in the 2nd category of the National Red List of protection in Belarus (Endangered/EN, according to the International Union for Conservation of Nature/IUCN system) (Качановский, 2015). It is a promising resource species for the needs of breeding and for the reclamation of cutting peatlands (Rapp *et al*., 1993; Campbell, Rochefort, 2003).

Within the framework of international research programs in Belarus, it is scheduled to work on the study of cloudberry genetic diversity and its chemical composition. The biochemical composition of cloudberries varies depending on the growth zone and climatic conditions (Hykkerud *et al*., 2018). Leaves have shown astringent, hemostatic, diuretic, and anti-inflammatory effects. In plant raw materials, mainly secondary metabolites have antioxidant activity. It has been experimentally shown that water-alcohol extracts of leaves and fruits have antioxidant properties. In a plant organism peroxidase is the most important substance with antioxidant activity. The level of peroxidase activity in assimilating organs was determined in samples from Belarusian populations. Peroxidase activity (PA) is a marker of stress and helps to determine the quality of a habitat. This message presents the interim results of the study.

Material and methods

Geobotanical studies covered the following coenopopulations: in Belarus - Narochansky National Park (NP), Lonno Wildlife Refuge (WR), Veliky Mokh WR, Yelnya WR.

Phytoindication of ecological regimes of the biotope was carried out according to the methods of Tsyganov (Цыганов, 1983) and Ellenberg (Ellenberg, 1996). The ecological valency to the abiotic factors of this population relative to the species range of ecological tolerance has been determined.

Population studies covered the ontogenetic structure of coenopopulations, morphometry of leaf blades, and accounting for the yield of female clones. An assessment of risks and negative impacts was made.

Biochemical research In biochemical tests, we used materials from Belarusian localities. The phosphomolybdenum method was chosen from a variety of methods for determining antioxidant activity (Shirwaikar *et al*., 2003). To determine peroxidase activity, enriched fractions of plant material were obtained by extraction with 0.02 M Tris-acetate buffer solution (pH 7.0) at the rate of 5 ml per a g of plant material. The extraction was carried out for 30 min at a temperature of 4 °C. The enriched fraction was separated by centrifugation at 6,000 rpm – 1 for 10 min. Protein concentration was determined by the method of Warburg and Christian and/or by the Lowry method (Леонтьев, Ахрамович, 2008). PA was determined spectrophotometrically. The optical density of the mixture containing the enriched fraction in a selected dilution, substrate (o-dianisidine), and hydrogen peroxide was measured with constant stirring, the automatic introduction of which triggered the reaction. A Specord 200 PLUS (Analytik Jena AG, Germany) spectrophotometer was used. The measurements were carried out at a wavelength of 460 nm. One ml of the solution was added to 1 ml of the various concentrations extract 0.6 M sulfuric acid, 1 ml 28 mM sodium phosphate solution, and 1 ml solution 4 mM ammonium molybdate. The lidded tubes were incubated in a thermostat at 95 ° C for 90 minutes. After cooling to room temperature, the optical density was measured on a Specord 200 PLUS spectrophotometer at 695 nm with respect to a blank sample. The activity was compared with ascorbic acid (1.7 mM solution) as a standard.

Total antioxidant activity was calculated using the following equation: % of total antioxidant capacity = ((Acontr-Ax) / Acontr) × 100%, where Acontr is the absorption of control.

Results and discussion

In Belarus the environmental conditions and the sexual structure of coenopopulations were determined. Among the Belarusian populations, the territory of the wet peatland forest around Lake Lonno was recognized as the most stable. The population Velikij Mokh is in the zone of post-pyrogenic transformation. Fruiting is observed in a small area not affected by fire. The population in Narochansky NP has a small area, females clones were not marked.

In the group of climatic factors at the sites of growth according to the thermoclimatic scale (TM), all localities belong to the mesoboreal suite, with the least

values being the Velikij Mokh bog. In terms of continentality (KN), the communities belong to the 1st continental formation. In the cryoclimatic (CR) range, localities are located at the boundary of the 1st and 2nd cryothermal formations. Ombro-regimes of all studied communities (OM) refer to the 1st humid zone. The salt regime was detected in all cases as glycosubmesotrophic, with the highest mineralization in the Lonno locality. The acidity of the substrates in all cases is subacidophilic 1. The localities differ insignificantly in the light factor (LC), with a tendency to decrease the light intensity in the Lonno locality.

Environmental and phytocenotic data were confirmed by results of biochemical analysis of Belarusian samples. The highest amount of protein (393 mg/g) and the lowest peroxidase activity ($2.956 \cdot 10^{-3}$ μmol / mg protein) characterize the Lonno population. The least amount of protein (335 mg/g) and the highest PA ($4.196 \cdot 10^{-3}$ μmol / mg protein) were observed in samples from the Velikij Mokh population, where plants were inhibited by post-pyrogenic transformation. Protein (363 mg/g) and PA indices ($2.853 \cdot 10^{-3}$ μmol / mg protein) of the third population occupy an intermediate position between the first two. AA is the absorption of the sample. Table 1 presents the results of studies of antioxidant and antiradical activity of leaves and petioles of cloudberry squat.

Table 1. Antioxidant activity (AA) of leaves and petioles of cloudberry, %

locality	Leaf blades*	Petioles
Lonno WR.	67.95±1.94	43.21±1.08
Narochansky NP	52.23±1.76	25.94±0.96
Veliky Mokh WR	47.07±1.05	18.62±0.34

Note. * For analysis, the extract was diluted 5 times.

The results show that the total AA of extracts of leaf blades is higher than that of petioles. The highest AA was observed in the northernmost and low illuminated coenopopulation, which consists of old, generatively active clones. The smallest AA is characteristic of the young population growing on the site of a burned-out area in an open highly illuminated area.

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A new eryophyoid mite (Acari: Prostigmata) in Latvia found on black currants

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Keywords: plant feeding mites, Eriophyoidea, *Ribes nigrum*

Eriophyoidea mites are known to be plant feeders and harmful to many plant species, as wild, as well as commercial (Ent, van der et al., 2017). Most of these species are quite specific for the host plant on which they feed, usually being confined to one plant species, or one plant genus, or to the members of a single family. These mites cannot survive for long periods away from their host plant, and, thus, most of the plant species on which they feed are perennials.

About 67 Eriophyidae species are known in Latvia, including mites harmful to plants of the genus *Ribes* (Stalažs, Turka, 2019). There are about 3-4 *Cecidophyopsis* spp. (Eriophyidae, Cecidophyinae, Cecidophyini) which are common on black currants *Ribes nigrum*, and of these, the most widespread and harmful species is *Cecidophiopsis ribis*. Black and red currant plantations in Latvia occupies about 1374 ha, and *C. ribis* is a common pest species there. Its presence is easily recognizable by the swollen black currant buds.

A sampling of damaged black currant leaves was made in commercial plantations in the eastern part of Latvia. The collected pest mite was supposed to be *C. ribis*. However, some doubt appeared as there were no swollen buds found on the black currants. Leaves with dead mites ("mummies") were sent to Jim Amrine (USA) for identification. The mites were determined to be *Anthocoptes masseei* (Nalepa, 1925), a new combination, previously listed as *Phyllocoptes masseei* Nalepa, 1925 or *Aculus masseei* (Nalepa, 1925). During the identification process, it was learned that the mite, *Phyllocoptes masseei* Nalepa, 1925 is actully the deutogyne form of this species and *Anthocoptes ribis* Massee, 1929 is the protogyne form of the same species (many males were found and all had the characteristics of *Anthocoptes*). Henceforth, by priority of publication date, the name *Anthocoptes ribis* Massee, 1929 becomes a junior synonym for the species *Anthocoptes masseei* (Nalepa, 1925). The two names, especially *masseei* Nalepa, 1925, have been placed in the genera *Eriophyes, Phyllocoptes, Anthocoptes, Aculus*, and *Vasates* by about 42 different authors.

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Investigation of genetic variants of immunoproteasome genes in Latvian population

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Abstract. Proteasomes are part of the basic mechanism by which cells regulate the concentration of certain proteins and degrade misfolded proteins, the so-called ubiquitin-proteasome system (UPS). UPS plays a crucial role in immunity and its dysregulation and/or modulation may influence different diseases. In most cells in 20S proteasome due to various conditions occurs replacement of three catalytic subunits: PSMB5, PSMB6, and PSMB1, by other subunits: PSMB8 (LMP7), PSMB9 (LMP2) and PSMB10 (MECL-1), respectively, and forms immunoproteasome. Immune system cells, especially antigen-presenting cells, express a higher basal level of immunoproteasomes.

The aim of the study was to determine the prevalence and possible functionality of SNPs of immunoproteasome genes and to analyse their usability as molecular markers in Latvian population. Literature and sequence data on three SNPs of immunoproteasome genes: *PSMB8* (rs2071543 and rs9357155) and *PSMB9* (rs17587) were analysed using meta-analysis, bioinformatic tools and genotyping of 208 DNA samples of healthy individuals. The frequencies of minor alleles (MAF) of the studied polymorphisms in the Latvian population are lower than the average MAF in Europeans. Most studied SNPs demonstrated allele-dependent alternative secondary structures, differences simulated DNA curvature and bendability and form/terminate the potential binding site of transcription factors in healthy individuals. Taking into account that there is a lot of literature data on investigated SNPs associations with different diseases, it can be assumed that the study of genetic variations in the *PSMB8* and *PSMB9* immunoproteasome genes, as possible molecular markers of autoimmune diseases, is a promising direction in further associative studies.

Keywords: proteasomes; SNPs; meta-analysis; bioinformatics; molecular markers.

Introduction

A proteasome is an evolutionarily ancient complex of enzymes that is also present in a simplified form in archaebacteria and it is evolutionarily similar between organisms (Groettrup et al., 2010). In eukaryotic cells, proteasomes are 700-1600 kDa protein complexes responsible for ubiquitin and/or ATP-dependent proteolysis (Budenholzer et al. 2017, Coux et al. 1996). Proteasome pathways play a central role in regulating a variety of protein functions by controlling not only their turnover but also the physiological behaviour of the cell (Lata et al., 2018). The ubiquitin proteasomal system is one of the major protein degradation pathways in the human body, accounting for more than 80% of protein degradation in eukaryotic cells (Im and Chung 2016).

A central proteolytic unit, known as the 20S proteasome, is a ~ 700 kDa complex consisting of four rings $\alpha_{1-7}\beta_{1-7}\beta_{1-7}\alpha_{1-7}$ with 14 different proteins (Im and Chung 2016). Together with the 19S regulator it makes up a 26S structure (Groettrup et al., 2010). Moreover, the interferon-γ (IFN-γ) inducible heteroheptameric regulator proteasome activator 28 (PA28), which is composed of PA28α (also PSME1) and PA28β (PSME2)

subunits, can associate with the 26S proteasome to form the 'hybrid' proteasome (Groettrup et al., 2010).

Under conditions of acute immune or stress response, or different inhibitors, three β subunits (PSMB5, PSMB6, and PSMB1) of 20S proteasome can be substituted during *de novo* proteasome biosynthesis with the interferon-γ inducible subunits β1i, β2i, and β5i, also known as PSMB9 (LMP2), PSMB10 (MECL-1) and PSMB8 (LMP7). This results in the replacement of standard 20S proteasomes with immunoproteasomes (Groettrup et al., 2010). Immune system cells, especially antigen-presenting cells, express a higher basal level of immunoproteasomes (Ferrington and Gregerson, 2012).

Mapping of the LMP2 (*PSMB9*) and LMP7 (*PSMB8*) genes to the major histocompatibility complex (MHC) locus on human chromosome 6 combined with interferon-induced activation first led to the proposal that the immunoproteasome carries out a specialized role in generating peptides for MHC class I antigen presentation (Ferrington and Gregerson, 2012). The presence of binding sites for multiple transcription factors, such as CREB and NF-κB on the promoter region of i-proteasome genes suggests that additional cytokine-independent mechanisms of regulation are possible (Ferrington and Gregerson, 2012).

Several studies have demonstrated that rs2071543 in the *PSMB8* gene and rs17587 in *PSMB9* might be associated with human diseases, including viral infections and cancers (Li et al., 2020). In 2002, it was found that rs2071543 of PSMB8 gene is associated with interferon response in patients with chronic hepatitis C, and it was presumed that rs2071543 have possible functional consequences (Sugimoto et al., 2002).

Therefore, a full understanding of the immunoproteasome requires an understanding of the role of these additional subunits and their gene polymorphisms. Our goal was to determine the prevalence of SNPs of *PSMB8* (rs2071543 and rs9357155) and *PSMB9* (rs17587) in Latvian population and its possible functionality as a change of DNA and RNA secondary structure, DNA bending and transcription factor binding sites, and to analyse their usability as molecular markers for future association studies with autoimmune diseases in our population.

Material and methods

Samples

A total sample group was formed of 208 healthy individuals from the Latvian population from the Latvian Centre for Marine Medicine, Vecmilgravis Hospital, and from Genome Database of Latvian Population, Latvian Biomedical Research and Study Centre (http://biomed.lu.lv/gene/*)*. No significant differences in genetic diversity were found between both sample sets.

All samples were carefully assessed to exclude the diagnosis of any disorders. The study was performed according to the Declaration of Helsinki and the protocol was approved by the Central Medical Ethics Committee of Latvia and informed consent was obtained from all participants of the study.

DNA extraction and genotyping

Genomic DNA was extracted from nucleated blood cells using a kit for genomic DNA extraction (Fermentas, Vilnius, Lithuania). Quality of DNA was determined using agarose gel electrophoresis, but quantity – with nano-spectrophotometry.

Basic PCR for two SNPs (*PSMA8* rs2071543 and *PSMB9* rs17587) and PCR with mismatched forward primer for *PSMA8* rs9357155 were performed with Dream Taq polymerase (Thermo Scientific, USA) using following parameters: 94°C for 5 minutes; then 35-40 cycles of 94°C for 45 seconds, appropriate annealing temperature for 45 seconds, 72°C for 45 seconds and a final extension step at 72°C for 7 minutes.

Table 1. Primers and genotyping method of SNPs.

Gene	SNP	Alleles^	PCR primer (from 5' to 3')	Restriction enzyme for RFLP	Sample size*
PSMA8	rs2071543	C/A	F: GAGCGGACAGATCTCTGGGTG R: TTCTTTGGGTCTGGCGCTC	*BsmI*	324/ 177+147
	rs9357155	C/T	MF:GGAGGGAGTAGGAGTATATG**C**TG R: TCTCTTTCCTCACTCCACCTT	*DdeI*	249/ 21+228
PSMB9	rs17587	G/A	F: GCTCCTCCAGCCTTTTCTGA R: CTCATGTAGGCTTGCGCCC	*HhaI*	252/ 41+211

^ - common/minor alleles; PCR – polymerase chain reaction; RFLP – restriction fragment length polymorphism; * Sample size of PCR product or no-cut RFLP fragment/cut RFLP fragments; F – forward primer; R – reversal primer; MF – mismatched forward primer.

All three SNPs were genotyped by restriction fragment length polymorphism (RFLP) analysis (Table 1). Oligonucleotide primers were designed using an online tool Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). PCR products were digested in a total volume of 10µl using restriction enzyme (Table1; 5 U/µl, Thermo Scientific, USA).

As sequence information for SNP localization and for oligonucleotide primers was used NCBI (https://www.ncbi.nlm.nih.gov/) reference sequence: NC_000006.12 (the chromosome 6 GRCh38.p12 assembly). Loci description and nucleotide numbering are given according to the recommended nomenclature system (http://www.genomic.unimelb.edu.au/mdi/mutnomen/recs.html).

Amplified and digested products were analyzed by electrophoresis in 1 – 3% agarose gel for all markers. For quality control, 16 randomly chosen samples per each marker were genotyped twice in different experiments. The concordance of the genotyping was 100%. Genotyping data were verified by direct sequencing of the corresponding DNA fragments using the Applied Biosystems 3130*xl* Genetic Analyzer.

Population analysis and meta-analyser

Information about each SNP on the distribution of rare alleles and genotypes was compared with data from different populations from the 1000 Genomes Project Phase 3 from Ensembl database (http://www.ensembl.org/Homo_sapiens/Info/Index) and with information from published studies involving the association or cohort study of the particular SNP.

SNP functional analysis in silico

An eventual functional significance of SNPs was analysed *in silico* on sequence similarity to transcription factors binding sites (TFBSs) using Genomatix software, MatInspector, Release 7.4 online tool (Cartharius et al., 2005) (www.genomatix.de). Only parameters with core/matrix similarity or more than 1.00/0.85 were considered. DNA secondary structures were predicted using the Mfold web server (Zuker, 2003) (www.bioinfo.rpi.edu/zukerm/cgi-bin/rna-index.cgi). Folding was simulated at 37^0C and

with 20 mM Na^{+} and 1.5 mM Mg^{++} for Intracellular or/and 145 mM Na^{+} and 0.5 mM $Mg^{++}$for Extracellular (Alberts, 2014). If various similar structures were obtained, structures with the highest negative free energy were representative. The possible effect of SNP on the DNA bendability was determined using the bend.it server (Munteanu et al., 1998) (hydra.icgeb.trieste.it/dna/bend_it.html).

Results and discussion

Population analysis

Analyzing the three SNPs in the two proteasome genes, it was found that in the case of both SNPs of the *PSMB8*, the frequency of a minor allele (MAF) in Latvian population (LV) is very different from the frequency of the average European population (EUR) (Table 2). The MAF was lower in LV than the average MAF in EUR.

Table 2. Summary of results of genotyping, bioinformatical and meta-analysis of studied SNPs.

SNP	Localization in genome (chr.6) with alleles# and in gene		Frequency of rear allele#		SNP possible functionality^				Meta-analysis
			In LV	In EUR	TFBS	DNS SS	RNA SS	DNA bending	
rs2071543	NM_004159.4: c.135+427C>A	1st intron or exon*	0.09	0.15	+/+	-	+	↓	Y
rs9357155	NM_148919.3: c.537+63C>T	5th intron	0.09	0.14	+/-	+	-	↑	N
rs17587	NM_002800.4: c.179G>A	3rd exon	0.25	0.26	+/+	+	-	-	Y

rear allele is second; * - PSMB8 has two transcription forms; LV – Latvian population (our study); EUR – average in European population according to1000 Genomes Project Phase 3 from Ensembl database; ^ TFBS - transcription factors binding sites determined using MatInspector, Release 7.4 (Cartharius et al., 2005)(www.genomatix.de), SS - secondary structures of DNS or RNA predicted with Mfold web server (Zuker, 2003) (www.bioinfo.rpi.edu/zukerm/cgi-bin/rna-index.cgi); "+" or "-" are/no changes between alleles; ↑ or ↓ increases or decreases in DNA bendability by the change of alleles. Y or N – is/no association with different diseases.

Genotypes containing rare alleles of the studied genetic variations, which in association studies are more often associated with various diseases, are less common in LV as compared to other European populations (data not shown). In the case of rs2071543, CA and AA genotypes carrying the minor alleles were detected with a frequency of 0.18 in LV and 0.27 in EUR; CT and TT genotypes of rs9357155, the frequency in LV was 0.17, but in EUR - 0.26. For rs17587 (*PSMB9)*, no such differences were found for genotypes and the frequency values were similar.

SNP functional analysis in silico

Analysing the possible SNP functionality, we looked at the formation of transcription factors binding sites (TFBSs), changes in DNA and RNA secondary structures (SS), and changes in DNA flexibility. The SNP in the exon or gene translational can affect protein replacement, as in the case of *PSMB8* rs2071543 (substitution from Glutamine to Lysine in 49th position of protein) if the second form of the gene is transcribed, and

also in the case of *PSMB9* SNP rs17587 (substitution from Arginine to Histidine in 60th position of protein).

However, if the SNP is located in an untranslated region - 5'UTR, intron, 3'UTR or between genes - it can affect other regions of the gene by altering the binding sites of transcription proteins or other elements, changing the nucleotide sequence or altering the DNA/RNA secondary structure or DNA bendability (Tak & Farnham, 2015; Sierra et al., 2020). Several studies suggest that approximately one-third of all SNPs that are not in the coding part of genes or do not affect amino acid substitutions are indirectly related to human health (Amir et al., 2019).

Nucleotide substitutions in the case of all studied polymorphisms cause changes in the binding sites for transcription factors. Thus, we can assume that the presence of one allele or another affects the properties of a particular region of the gene in different ways.

In addition, we found that *PSMB8* SNP rs2071543 proposes changes in RNA SS form, which could be explained by the fact that the SNP affects the amino acid change (Shen et al., 1999). In the case of the other two SNPs, a change in the secondary DNA structure occurs. Thus, it can affect the binding of different elements to a DNA sequence to activate or, conversely, deactivate different processes.

Various SNP nucleotides can affect the structural properties of DNA, such as bendability and elasticity, and thus play an important role in DNA recognition by sequence-specific DNA-binding proteins (Dlakić et al., 2005). Analysing the DNA bendability in each SNP region (Table 2), we concluded that in the case of *PSMB8* SNPs: rs2071543 and rs9357155, the bendability decreases and increases, respectively, in the case of the minor allele, but in the case of PSMB9 SNP rs17587 no possible changes were identified.

Meta-analysis of SNPs

The final point in our study was to check whether specific SNPs were being studied for association with different diseases or with human health-related symptoms in different populations.

PSMB8 SNP rs2071543 and *PSMB9* SNP rs17587 have been associated with various diseases (Table 2), for example, *PSMB8* SNP rs2071543 has been associated with response to interferon-beta therapy in hepatitis C patients in the Japanese population (Sugimoto et al. 2002) and in multiple sclerosis patients in the Irish population (Cunningham et al. al. 2005). But in the case of *PSMB9* SNP rs17587 the associations have been found, for example, with female patients with multiple sclerosis in the Italian population (Mishto et al., 2010) and with type 1 diabetes mellitus in the North Indian population (Saida et al., 2014).

A bioinformatic and meta-analysis of the selected SNPs for *PSMB8* (rs2071543 and rs9357155) and *PSMB9 (*rs17587) illustrates the possibility of their use as possible molecular markers of autoimmune diseases and is a promising direction for further association studies.

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Ecological quality of lake Lizdole

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Abstract: The usefulness of living organisms to detect environmental changes has frequently been confirmed in terrestrial as well as aquatic ecosystems. Many groups of organisms are useful indicators of the presence and concentration of pollution, especially trophic pollution. The objectives of our survey were to assess the water quality of lake Lizdole using several biological parameters (phytoplankton, macrophytes, macroinvertebrates, and zooplankton) and physico-chemical parameters.

Keywords*: biological quality elements, lake stratification, physico-chemical parameters*

Introduction

The value of healthy aquatic ecosystems is inestimable because they provide many ecosystem services such as purification of water, decomposition of organic matter, and others, which we usually take for granted, and biodiversity is one of the most threatened resources. The study by Grizzetti et al. (2019) demonstrates that aquatic ecosystems of good ecological status can provide more regulating and cultural ecosystem services.

In Europe, about 40% of all the surface waterbodies are at good or high ecological status, and in Latvia only 20% of lakes and rivers are at least in good ecological status (EEA, 2018), meaning that more efficient management of waterbodies is required.

Lake Lizdole is a shallow brown-water lake with high water hardness (lake type L6) in good ecological status. Lake catchment area is 2.4 km^2 and out of that 64% are occupied by forests. According to measurements done in 2019, the lake surface area is 54.7 ha, average depth – 4.1 m, and maximum depth – 12.7 m. There are no intensive human activities, except the NE shore of the lake, where a recreational base "Silmači" is located.

The aim of this study was to assess the ecological status of Lake Lizdole according to different biological and physico-chemical parameters.

Material and methods

Surveys of Lake Lizdole were carried out in June, August, and October 2019. During the surveys, in-situ measurements of water temperature, pH, conductivity, and dissolved oxygen were carried out. Water samples for analysis of nitrogen and phosphorus compounds were taken at a depth of 0.5 m. The analysis was performed in an accredited laboratory.

Phytoplankton samples were collected at a depth of 0.5 m and preserved in Lugol solution. Phytoplankton cells were counted with the Leica DML inverted microscope. Biovolumes were calculated by comparing cells to simple shapes and applying standard geometric formulae (Utermohl 1958).

Zooplankton samples were collected in August of 2019 by filtering 100 L of lake water through the Apstein net with a mesh size of 55 µm. Zooplankton samples were preserved in 4% formalin solution. Quantitative and qualitative analyses were conducted on 1 cm^3 sub-samples. Biomasses were calculated by comparing organisms to simple shapes and applying standard geometric formulae (Bottrell et al. 1976).

Macroinvertebrate samples were collected in October of 2019 from the littoral zone using a kick net (frame size 25x25 cm, mesh size 0.5 mm). Samples were further processed at the laboratory and macroinvertebrates were identified to the nearest achievable taxonomic level. Lake ecological quality was assessed by using LLMMI (benthic invertebrates) index (Skuja and Ozoliņš, 2016).

The macrophyte survey was done in August of 2019. Macrophyte taxa were determined to species level where possible, except for some large benthic algae which were determined to the genus. Macrophyte species and their abundance were recorded in 3 transects. Within each transect, all macrophyte species and plant communities of submerged, floating-leaved, and emergent vegetation were recorded and their abundance was estimated according to a seven-point scale. The total macrophyte cover of the lake surface was estimated.

Results and discussion

Physico-chemical parameters

According to chemical parameters, Lake Lizdole has a moderate ecological status due to elevated total P concentrations (0.057 – 0.090 mg/L). Dissolved P-PO_4^{3-} concentrations are considerably lower (0.011 – 0.034 mg P/L) indicating that the largest P fraction is particulate P. Average total N concentrations are in the range 0.52 – 0.61 mg/L, which corresponds to good status. Average N-NO_3^- concentrations are low (<0.07 mg/L), and N-NH_4^+ are in range 0.007 – 0.043 mg/L.

During summer stratification, the lake thermocline is in the depth of 3 – 7 m. O_2 concentration is rapidly decreasing in the thermocline and at 5 m depth, it drops below 0.18 mg/L (1.7 % saturation) (Figure 1). Such a distribution pattern of dissolved oxygen is typical for eutrophic lakes.

Phytoplankton

According to phytoplankton composition, biomass, and the number of organisms, Lake Lizdole corresponds to good ecological quality. Phytoplankton composition and biomasses are similar both in pelagic and littoral sampling stations. Total phytoplankton biomass in summer reaches up to 0,4 mg/L. In August samples, Cyanophyta are dominating, however, potentially toxic species *Oscillatoria sp.*, *Anabaena sp.* and *Aphanizomenon flos-aquae* were in very low concentrations. The biomass of *Microcystis sp.* was low.

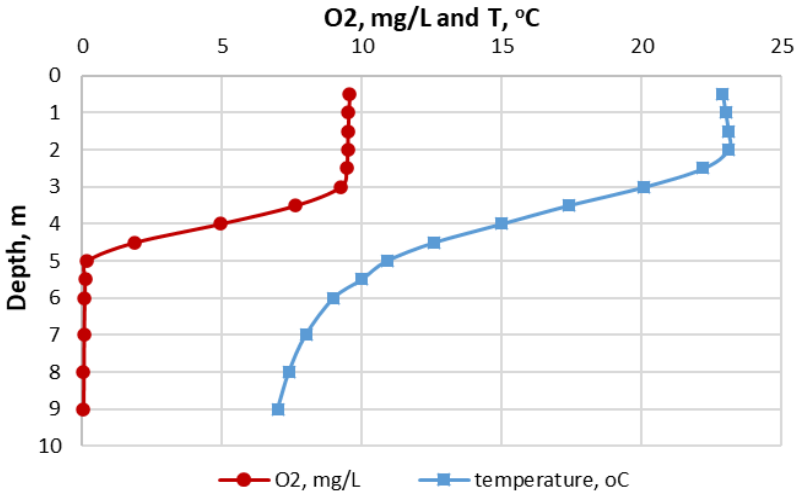

Figure 1. Stratification pattern in Lake Lizdole (June 2019).

Macrophytes

A total of 20 plant taxa were recorded. The most frequent plants were *Myriophyllum verticillatum*, *Potamogeton lucens*, *Myriophyllum spicatum,* and *Chlorophyta*. Among the taxa found in the lake, there were two rare and protected species – *Myriophyllum alterniflorum* and *Nuphar pumila*. Both species are included in the Red Data Book of Latvia. Total coverage of macrophytes was 30%. The largest overgrowth was found in the northern bay of the lake, where dominated *Myriophyllum sp*. and *Chlorophyta.*

Zooplankton

In Lake Lizdole, the overall abundance of zooplankton is 22 thousand ind/m^3. The dominant taxa are Copepoda (52%), while Rotatoria forms 27% and Cladocera - 21% of the community. Rotatoria is represented by several species though *Pompholyx sulcata* is one of the dominant taxa indicating eutrophic conditions. Also, *Trichocerca capucina* and *T. cylindrica* indicate eutrophication, however, their abundance is relatively low. In the suborder Copepoda, juveniles of Cyclopoida are dominant, indicating eutrophication as well. In the order Cladocera, eight taxa were observed with dominant species *Bosmina coregoni, B. longirostris,* and *Daphnia cristata*. According to the taxonomical composition of zooplankton, Lizdole Lake is eutrophic.

Macroinvertebrates

In the Lizdole Lake, altogether 36 different macroinvertebrate taxa were observed. The dominant taxa are larvae of insects, especially caddisflies Trichoptera. According to macroinvertebrates, the ecological quality of Lake Lizdole is good (LLMMI in 2019 = 0.65).

Acknowledgements

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The effect of intake of red beetroot juice fractionated by ultrafiltration method on physical strength and endurance of laboratory rats

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Abstract: The creation of innovative technologies and products is an actual problem for agriculture, food, and human health. The use of local natural resources is important for the development of the national economy and medicine. Red beet is one of the most widely used vegetables in Latvia. In the present study, the influence of the administration of developed fractionated red beetroot juice (RBRJ) on physical activities in trained rat was investigated. Assessment of experimental data showed the beneficial effect of RBRJ that resulted in rat physical strength and endurance improvement for one-month experimental training. It can be concluded that the use of the product is promising.

Keywords: red beet, ultrafiltration, body weight, muscle, blood, biochemical indices

Introduction

Last time there has been growing interest in the impact of red beet *(Beta vulgaris*) root on human health. The beneficial effect of red beetroot juice (RBRJ) on people has long been known. RBRJ consumption provides the possibility to modulate many functions of mammal organism (Hamedi S. & Honarvar M., 2018). The use of such juice, due to its valuable chemical composition, reach in iron, vitamin C, antioxidant pigments, helps to improve the functioning of the gastrointestinal and cardiovascular systems and also provides enhance of energy stamina in mammals (Webb A.J. *et al*., 2008).

To increase RBRJ specific activity a method of fractionation of the juice based on molecular weight ultrafiltration was applied at the experiment (Babarykin D., *et al*., 2018).

The present study was conducted to evaluate the effect of fractionated red beetroot juice (RBRJ) on physical strength and endurance of rats trained in laboratory experiments.

Materials and methods

Ethics Statement

All experimental procedures were approved by the Animal Ethics Committee of the Food and Veterinary Service (Riga, Latvia, authorisation reference number 53, (April 10, 2012).

Material

Deproteinized fractionated RBRJ from red beet *(Beta vulgaris)* root was obtained on the laboratory equipment for juice fractionation by molecular mass using „Ultracel”

membrane for ultrafiltration of beet juice (Babarykin D., *et al*., 2018). Laboratory male *Wistar* rats were used for the investigation.

Experimental design and animals

The experimental animals were 5-week-old with bodyweight of 120 g. 20 rats were randomized into two groups of 10 heads in each. Group 1 (control trained) – healthy rats, which were fed a standard rat chow and were running on a treadmill 5 days a week during one month. Group 2 (trained group + fractionated RBRJ) – healthy rats, which were fed the same standard rat chow and 2 h before exercises additionally were administered 0.5 ml fractionated red beetroot juice orally daily. Animals were trained in a motorised wheel with gradual speed increase for 4 weeks. Muscle strengths (by electronic dynamometry) and endurance of rats using its electrical stimulation on a racetrack once a week.

At the end of the experiment, biochemical blood indices were analyzed, adipose tissue from the abdominal cavity was collected and weighted, and parameters of *gastrocnemius* muscle mass and animal body weight gain were determined.

Statistical analysis

All statistics were performed using the program SPSS. Means and Standard deviations and significance values were calculated. Results are presented as mean ± SD. Statistical comparisons were performed using Student's t-test. Statistical significance was set at $P < 0.05$.

Results and discussion

Weighting the animals once a week during one month of the experiment did not show the significant effect of *per oral* intake of fractionated RBRJ on body weight in trained rats (Figure 1).

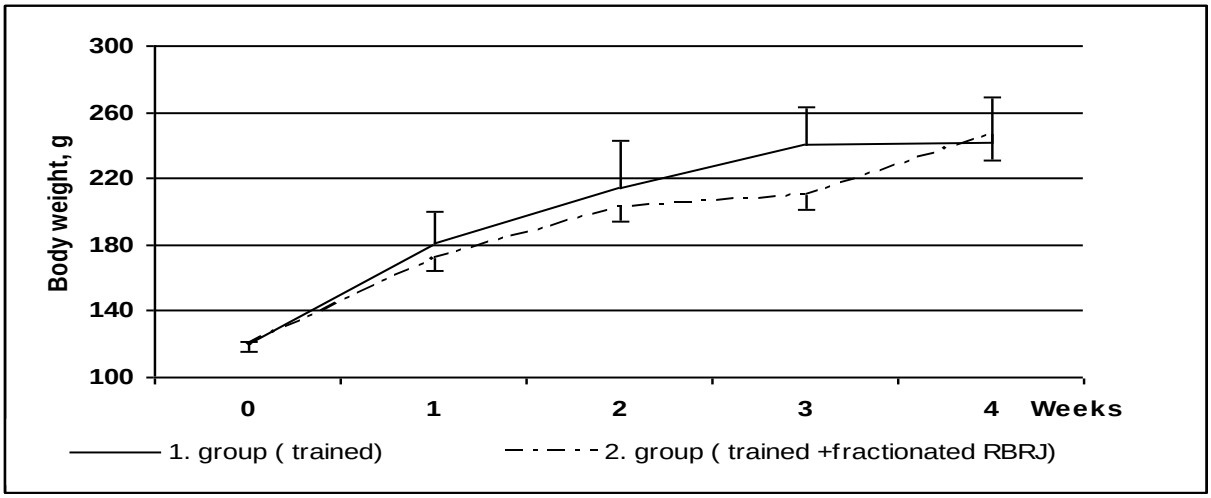

Figure1. The effect of fractionated red beetroot juice intake on rat body weight for the experimental period

The observed experimental weight data of rat leg muscles compared with body mass are presented in Table1.

Table1. The effect of fractionated red beetroot juice intake on mass changes of calf and adipose tissue in rats

Group of rats	*Mm. Gastrocnemius* weight, g	*Mm. Gastrocnemii* relative weight comparing with body weight, %	Peritoneal adipose tissue weight, g	Peritoneal adipose tissue relative weight comparing with body weight, %
Group 1 (control trained)	0.73±0.26	0.61±0.21	4.4±1.6	179±0.25
Group 2 (trained + fractionated RBRJ)	1.15±0.05*	0.93±0.05*	3.04±1.0	129±0.12*
*Statisically different from control (P<0.05)				

Administration of fractionated RBRJ in rats during one month of physical training along with a growth of *gastrocnemius* muscle mass (about 50%), showed a decrease of specific weight of peritoneal adipose tissue by 30%.

Table 2 demonstrates the influence of RBRJ intake on physical endurance in trained on a motorized rats.

Table 2. The effect of fractionated red beetroot juice intake on physical parameters in trained rats

Parameter	Group 1 (control trained)	Group 2 (trained + fractionated RBRJ)
Body weight gain, g	122.7±27.8	128.4±18.9
Muscle strength, g Front paws All paws together	 291.4±14.7 563.0±27.9	 353.3±25.8 * 671.4±32.6 *
Physical stamina (the number of the rat falls from motorized racetrack)	60.0±17.7	23.0±6.8 *
* Statistically different from control (P<0.05)		

It was observed a slight decrease in animal body weight and the increase of paw muscle strength after RBRJ intake. Moreover, the significant enhance of physical strength of animal extremities also was determined. Physical stamina of rats administered by RBRJ increased almost 3 times.

Analysis of biochemical parameters in rat blood did not reveal significant influence of RBRJ intake on determined indices (Table 3).

Table 3. The effect of fractionated red beetroot juice intake on blood biochemical indices in trained rats

Parameter, mmol/L	Group 1 (control trained)	Group 2 (trained + fractionated RBRJ)
Lactate	6.86±0.76	5.88±0.45
Cholesterol	2.19±0.33	2.22±0.30
High density lipoprotein	1.26±0.17	1.29±0.14
Low density lipoprotein	0.39±0.14	0.45±0.13
Triglycerides	0.74±0.24	0.75±0.20
Glutamate oxidase	6.51±0.99	5.11±1.03
Urea	7.01±0.45	5.29±0.65*
Creatinine	0.06±0.02	0.04±0.00
Fe	0.061±0.012	0.055±0.011
* Statistically different from control (P<0.05)		

The observed decrease of protein metabolism indices (urea and creatinine) may be associated with increased physical activity of experimental animals. Moreover, a reduced value of the lactate level in rats administered the fractionated RBRJ may indicate a better supply of oxygen to tissues.

The results of the present investigation show that fractionated red beet (Beta vulgaris) root juice is prospective for use as a functional food for athletes, as well as to improve the quality of life of persons suffering from functional insufficiency of the cardiovascular system, as well as in gerontology.

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Wind turbines and protection of bat species. Is "green energy" always green?

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Keywords: bats, mortality, wind turbines, impact mitigation

Wind power is usually considered a green form of renewable energy and is one of the fastest growing energy sources world-wide. Wind energy development is not, however, environmentally neutral and impacts on bats and other wildlife are of increasing concern. Wind energy development may affect bats in two primary ways: direct mortality impacts on individuals and indirect impacts via destruction or changes in the habitats (Barré *et al*., 2018; Rydell *et al*., 2010a,b). Currently, there is evidence that 27 European bat species suffer collision fatalities at wind turbines (Rodrigues *et al*., 2015). At the same time, all European bats are strictly protected under the EU Habitat Directive and the Bern Convention on Migratory Species, and killing even individual animals is strictly forbidden by law in all European countries.

There are several ways how to mitigate the impact of wind turbines on individual bats or their populations. They include a correct choice of the turbine location through pre-construction impact assessment, seasonal or other specific limitations of turbine operation, so called “feathering” method, elaboration of ultrasonic or light-based repellants etc. (Rodrigues *et al.,* 2015). All of these methods have their advantages and drawbacks; unfortunately, there is no “perfect” method developed so far that could guarantee a “close to zero” bat mortality. Objective limitations also exist during the pre-construction impact assessment, for example, bat presence in some areas may considerably increase only after the construction of turbines (Lintott *et al*., 2016). That may result in incorrect decisions for impact mitigation. Moreover, wind farm developers do not always agree with the proposed methods for bat species protection as they usually involve some economical losses.

In Latvia, the knowledge on bat mortality caused by wind farms is scarce. A pilot study in 2013 and some occasional observations as well as the presence of important migration routes that cross the territory of Latvia suggest that bat mortality can be high at some turbines at least during the migration period. Fatalities of four bat species *Eptesicus nilssonii*, *Pipistrellus nathusii*, *Pipistrellus pygmaeus* and *Vespertilio murinus* have been registered. Three of these species are long-distance migrants. Therefore, the current increase of wind energy development in Latvia gives reason for serious concern, because it could seriously jeopardize the long-term survival of the migratory bat populations in northern Europe.

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A review of common pochards (*Aythya ferina*) breeding distribution and population changes in Latvia (1958-2019)

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Keywords: nesting success rates, daily survival rate, predatory pressure

The assessment for the European Red List of Birds 2015 has indicated that in the last 20 years there have been serious reductions in the distribution and abundance of breeding common pochard, resulting in the European population being upgraded from IUCN *Least Concern* status to *Vulnerable* (BirdLife International 2015, IUCN 2020). For this reason, in countries included in the Agreement on the Conservation of African-Eurasian Migratory Waterbirds by the end of 2019 this species has been crossed from the list of game animals (MK noteikumi 2019).

Here, we review local population and distribution changes of common pochards in Latvia by using data from two long-term breeding duck surveys – in Lake Engure (1958–2019) and in Lake Kaņieris (2005–2019) – and breeding bird atlas data of three periods: 1980–1984 (Priednieks et al. 1989); 2000–2004 and 2013–2017 (Latvian Ornithological Society, unpubl.). Survey data were analysed by using two methods – apparent nest success (Lake Engure, Kaņieris) and daily survival rate (only for lake Kaņieris). For distribution comparison we used chi-squared test method (maps of all three atlases unified to 10×10 km grid).

Our results indicate that in Lake Engure nesting success rates for this species have decreased significantly (r^2=0.21, p<0.001) with the steepest decline registered by the end of 1990s. The overall numbers of nests found each season also have decreased dramatically in both island and emergent vegetation sample plots (from 131 nests in 1992 to just 6 nests in 2019). Meanwhile, nest survival rates for Lake Kaņieris during last 14-year period have stayed stable (apparent nest success: r^2=0.03, p<0.6, n=322*;* daily survival rate: r^2=0.81, p<0.2, n=178). Our atlas analysis results show that for this species in the last 33 years the overall distribution in Latvia has decreased significantly, with the largest decrease being between 1980- and 2000- year period (x^2=24.15, p<0.001).

This decrease of population distribution for pochard in Latvia is similar to the overall distribution changes in Europe and are more severe than modelled by the climatic atlas of European breeding birds (Huntley et al 2007), indicating that multiple environmental factors are at play. For the local population of Lake Engure the main cause of decline is a combination of different factors with the most probable being habitat loss, decrease of black-headed gull *Larus ridibundus* colonies, as well as increase in predatory pressure (increase of American Mink *Mustela vison* population). All these factors could be assessed to be affecting breeding common pochards in Latvia in general and therefore must be taken into consideration when planning how to increase this species conservation status.

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Level of methylation of promoter of the *petE* gene is not related to formation of green plants *in vitro*

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Keywords: barley, anthers culture, Cu ion-containing protein, epigenetics effect

The low production of green plants-regenerants is one of the problems of obtaining doubled haploids from cereals: the number of albino plants is significantly higher than the number of green plants-regenerants. The presence of copper ions (Cu) in the media during *in vitro* cultivation promotes formation of green plants in barley anther culture, nevertheless still proportion of albino plants-regenerants is much higher. Synthesis of Cu ion-containing protein - plastocyanine is controlled by nuclear gene *petE*. It can be hypothesized that even if Cu ions necessary for the synthesis of plastocyanine are available in the medium, transcription of the gene and, as a result, synthesis of the protein *in vitro* conditions is blocked by the methylation of the *petE* gene promoter, what, finally, is manifested in developing albino plants.

Two barley (*Hordeum vulgare* L.) doubled haploid lines with previously established contrasting genotypic response to embryogenesis were selected for experiment. For viable plants level of methylation in the region of the promoter of the *petE* gene was determined in different stages of plant development (germinated mature embryos, etiolated and green seedlings) of both genotypes. In anther's cultures the level of methylation was determined for pollens, induced embryos and for leaves of albino plants-regenerants.

For detection of methylation level unmethylated cytosine nucleotides of DNA were converted to uracil by using *Qiagen EpiTect Plus Bisulfite Conversion Kit* followed DNA purification by *Qiagen EpiTect Plus DNA Bisulfite Kit*. Purified DNA was performed by *Qiagen PyroMark PCR Kit* and finally to detect of methylation level of target DNA sequence was used *Qiagen PyroMark Q24 Advanced CpG Reagents* on *Qiagen PyroMark Q24 Advanced* equipment. Primers for PCR performance and pyrosequencing analyses were designed by *PyroMark Assay Design 2.0* software.

The collected results showed that the methylation level of *petE* gene promoter region in viable plants, pollens, from anther cultures obtained embryos and plants-regenerants was similar and allows conclude that formation of green plants in *in vitro* cultures is not related with methylation level of promoter region of *petE* gene.

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